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13. ABSTRACT ***(Maximum 200 Words)***

Contrast-enhanced breast Magnetic Resonance Imaging (MRI) is almost 100% sensitive for breast cancer, but is used rarely because false-positive MRI scans lead to unnecessary biopsies. Magnetic Resonance Spectroscopy (MRS) is a novel, noninvasive method that measures a functional breast cancer byproduct, choline, which might reliably distinguish cancer from benign disease. The purpose of our 3-year research program is to test the hypothesis that MRS distinguishes cancer from benign tissue. The scope of work includes identifying abnormal breast lesions on contrast-enhanced breast MRI, performing *in vivo* MRS to detect choline in the abnormal lesion, and obtaining cells from the tumor by needle biopsy to validate the presence of choline by a high-resolution *in vitro* spectroscopy scan in a chemistry laboratory. In our first year we have developed, optimized and tested *in vivo* and*in vitro* MRS scans in breast phantoms and patients using these methods, showing promising results for a novel multi-voxel MRS technique that encompasses large portions of the breast. We are now ready to initiate clinical testing in women with benign disease and breast cancers. The success of this pilot project would provide a noninvasive, independent method of distinguishing breast cancer from benign disease at the time of MRI scanning, making this powerful tool more widely available to all women.

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INTRODUCTION

Breast Magnetic Resonance imaging (MRI) using intravenous contrast detects breast cancers with almost 100% sensitivity by bright contrast enhancement in cancers [1-5] while normal tissue and scars older than six months do not enhance [2]. However, some benign entities also enhance, which limits the clinical usefulness of breast MRI. Thus, breast MRI is not widely used because of fears of false positive studies which would result in unnecessary breast biopsies, and underlines the need for a noninvasive tumor-specific MRI-compatible test to characterize MRI-detected breast lesions. *In vivo* magnetic resonance spectroscopy (MRS) is a noninvasive, innovative and increasingly widely used tool for MR tumor characterization [6-8], with choline-containing compounds emerging as one of the most important signals from cancers. The choline peak in an *in vivo* MR spectrum occurs at 3.2 ppm and reflects total cellular choline stores including choline, phosphocholine, phsophatidylcholine, glycerophosphocholine molecules, with some contributions from other choline-containing compounds [7,9]. An increase in the intensity of the 3.2 ppm resonance (hereafter this peak will be simply referred to choline) is widely believed to reflect increased membrane synthesis and/or an increased number of cells, indicating cancer [10-14]. These prior breast MRS studies imply that the *in vivo* proton spectra acquisition could be a powerful, innovative tool for enhancing lesions seen on breast MRI (15-17). Successful MRS development would be a noninvasive and powerful solution to the MRI dilemma of non-specificity. However, *in vivo* proton spectroscopy of tissues outside of the brain is technically very difficult to perform due to significant motion artifacts and large unwanted lipid signals that obscure the resonances of interest. One of the key questions to answer is whether MRS can be developed to detect choline peaks in MRI-enhancing cancers reliably and reproducibly while reducing motion artifact and suppressing lipids. Our work prior successfully measured creatine, choline, and lactate levels in numerous head and neck tumors [18-21], and our current work involves extent of these techniques for *in vivo* breast spectroscopy.

The **specific aims** of our study are:

1. To develop and optimize a new *in vivo* multi-voxel proton MRS technique to measure choline peaks in breast cancer. In year 1, the pulse sequence was optimized for detecting choline in breast tissue, with emphasis on eliminating water and lipid signals, and motion-induced artifacts. During this development and optimization phase, the pulse sequence was tested in phantoms and patients, and the *in vivo* cancer data was validated using both histology and high resolution *in vitro* MRS of tumor cells.

2. To demonstrate that *in vivo* MRS can distinguish cancer from benign breast disease. We hypothesize that *in vivo* MRS choline measurements will be elevated in breast cancer compared to benign tissue. The optimized MRS pulse sequence developed in Specific Aim 1 will be used in patients with cancer and patients with benign disease in years 2 and 3. Results will be compared with both histology and *in vitro* MRS of benign and malignant tumor cell samples. A t-test will be used to determine if *in vivo* choline levels are statistically different in the cancerous and benign lesions.

BODY

Our work was divided into a 3-year study, which was separated into two phases: (Phase1) technical development in year 1 corresponding to Specific Aim 1 and (Phase 2) clinical testing in years 2 and 3 corresponding to Specific Aim 2. During year 1 we planned for MRS technical development and optimization to:

(1) Develop and optimize high resolution *in vitro* MRS in phantoms
(2) Develop and optimize the *in vivo* MRS pulse sequences in phantoms
(3) Recruit and study normal volunteers and 5 breast cancer patients to further test and develop the optimized *in vivo* MRS pulse sequences and the high resolution *in vitro* MRS of tumor cells obtained by fine-needle aspiration on cancer patients.

Develop and optimize the *in vivo* MRS pulse sequences in phantomsIn years 2 and 3 we plan to evaluate our optimized MRS methods by studying patients with breast cancer and benign disease, and correlate the results with histology and high resolution *in vitro* MRS of tumor cells.

Our most significant accomplishments to date include development and optimization of the MRS pulse sequence for *in vivo* MR spectroscopy, development and optimization of the *in vitro* MR spectroscopy methodology, testing of both the *in vivo* and *in vitro* high resolution spectroscopy scans on phantoms, and later, on patients and on fine-needle aspiration (FNA) breast biopsy cell samples.

Our personnel infrastructure changed on the departure of Josh Star-Lack Ph.D. from Stanford University. Sandeep Hunjan, Ph.D., replaced Dr. Star-Lack in October 1999 to develop and optimize *in vivo* MR spectroscopy methodology for choline imaging in breast cancer, and to optimize the parameters for the high-resolution *in vitro* MRS scans of phantoms and breast biopsy samples. A second important addition was the hiring of Leslie Jerome-Roche, R.N as nurse coordinator to optimize contacts with referring physicians and to help with patient recruitment. Detailed aspects of this work are described below.

***In vitro* MR spectroscopy development, optimization and testing**: We developed a choline, creatine, and NAA phantom for *in vitro* high resolution MRS to optimize parameters for acquiring high resolution spectra in the wet laboratory. MR spectroscopic experiments were performed at 400 MHz or 9.4 T equipped with a Varian console and a 5-mm probe. Spectra were obtained with good signal to noise ratios (SNR) in 4 acquisitions with this phantom.

To obtain breast cell samples for the high resolution MRS *in vitro* studies in patients, informed consent was obtained to perform needle biopsies after the MRI/MRS scans. Patients were accompanied to the hospital immediately after the MRI / MRS scans. In the hospital, the breast lesions were biopsied with a fine needle aspiration (FNA) technique under direct ultrasound guidance after injection of local anesthesia and using sterile technique. Each biopsy obtained breast cell samples using sterile 21 gauge needles, and usually 4 samples were obtained with four separate needles. Alternatively, within 6 weeks of the MRI / MRS scan, cells were obtained from the mass in the operating room by fine-needle aspiration under direct palpation of the operated specimen, and again,

usually 4 samples were obtained with four separate needles. FNA samples were immediately placed into polypropylene vials that contained chilled phosphate buffered saline (PBS) in D2O. All specimens were then immediately immersed into liquid nitrogen and stored at –78 degrees C for up to 6 weeks until MR spectroscopic analysis could be performed. One sample was stored for 7 weeks. Before *in vitro* 1H MRS, each FNA sample was thawed and transferred to a 5-mm MR Spectroscopic tube under a vented hood in a wet laboratory. The volume was adjusted to 400 ul with PBS in D20. The samples were spun at 20-25 Hz, and the temperature was maintained at 37 degrees C. Residual water signal was suppressed with selective gated irradiation by using the decoupler placed on the water resonance. Proton spectra were acquired with the following parameters: sw = 5000 Hz, pw = 29 us (90 pulse), 256 free induction decays, acquisition time = 1.14 s and relaxation delay = 2 s (TR = 3.14 s). MR spectra of FNA specimens were processed using "NUTS" shareware running on a PC using MS Windows. A 3 Hz line broadening was applied to the data before Fourier Transformation followed by phase and baseline.

***In vivo* MRS pulse sequence development and optimization:** As part of our efforts to improve our ability to collect proton spectra from the breast in patients for the *in vivo* portion of the experiment, new pulse sequences designed to more robustly separate choline and lipid were developed and evaluated in phantoms. MRS pulse sequence development also included automated shimming. We redesigned the RF pulses for improved lipid and water suppression using dual BASING techniques. During pulse sequence development and testing in volunteers and in patients, we encountered considerable artifacts due to B1 and B0 inhomogeneities near the chest wall and heart, and in large patients where the breast touched the RF coil, resulting in "hot spots". To correct these "hot spots", we added highly selective saturation bands that were used to suppress these tissues giving rise to the artifacts. The resulting diagnostic MRI sequence and optimized MRS sequence resulted in a 90 minute scan time, requiring an overall 2-hour scan time slot for scan set-up, scan completion and archiving.

Testing and further development of the optimized *in vivo* MRS pulse sequences and the high resolution *in vitro* MRS of tumor: We developed and implemented a program and protocol to identify and recruit appropriate breast cancer patients from the Surgery, Oncology and Radiation Oncology departments using a nurse-coordinator, Leslie Jerome-Roche, RN. We used the pre-tested, optimized diagnostic MRI and MRS scans on subjects after informed consent (approved by the Stanford University IRB). All MRI and MRS examinations used a four channel phased-array breast RF coil (MRI Devices) and all scans were obtained on a conventional 1.5 T GE Signa scanner. Patients were initally scanned with an MRI pulse sequence to locate the lesion employing a high resolution three dimensional spectral spatial magnetization transfer pulse sequence (3DSSMT) [22-24] which provides high resolution morphologic data immediately following a rapid 3D spiral sequence which produces 3.5 minutes of kinetic data of breast lesion enhancement 3D spiral scan parameters included 20-interleave spirals, 20 cm FOV, TR/TE 38/11.9 ms, 500 flip angle, 4.5 to 6 mm thick slices, 188x188 matrix, 20 slices volume, scan time of 10.86s. 3DSSMT parameters included TR/TE 33-40/7 ms, 512x192 matrix, 60 slices, scan time of 1.0-2.0 min., using centric phase encoding, water selective excitation and on-resonance magnetization

transfer pulse [25]. In cases in which intravenous contrast could be used, we used 1 mmol/kg intravenous gadolinium given as a bolus during the initial spiral sequences and flushed immediately with 20 cc of sterile saline.

To obtain the MRS spectra we used spiral k-space trajectories (hereafter called spiral CSI [20]) with which we obtain both volumetric metabolite data and a rapid water reference acquisition used to automatically phase and quantify the spectroscopic data and extended spiral CSI to allow the acquisition of spatially resolved 2D NMR spectra. Details of a J-resolved spiral spectroscopic imaging sequence is given in [26]. The data processing for these spectroscopic studies is based on our previous work on signal estimation and water referencing using prior knowledge [27, 28].

We initally used a single-voxel MRS acquisition technique known as point resolved spectroscopy (PRESS [29]) on our first patients. The PRESS technique consists of three slice-selective excitation pulses along orthogonal axes, using presaturation for water suppression[30]. Our improved PRESS sequence using spectral-spatial excitation pulses achieves a significantly improved uniformity of metabolite intensities within the PRESS box along with decreased contamination by signals outside the volume of interest [31]. In addition, we achieved improved water and lipid suppression using an independently developed method known as band selective inversion with gradient dephasing (BASING[32]]. We acquired *in vivo* spectra of choline resonances by developing a two-shot J-difference single-voxel editing technique incorporating the BASING pulse sequence into PRESS localization. The technique requires two acquisitions in which the lactate methine quartet is inverted by the BASING pulses in the second acquisition but not the first. Sum spectra then yield the uncoupled singlets, including creatine, choline, and lipids, while difference spectra yield the lactate methyl doublet (1.3 ppm). Motion artifacts are minimized by tracking the frequency and phase of the residual lipid peaks. This artifact reduction technique results in lipid suppression factors of over 1000.

As part of our overall MRS program, 9 patients with suspicious breast masses or mammographic findings were studied with high-resolution breast MRI examinations (7 contrast-enhanced, 2 non-contrast) and MRS during the past year. As part of the Stanford MRS program, single-voxel scans were obtained on the first patients as part of the program to develop multi-voxel sequences used for this study; of 5 patients undergoing the multi-voxel MRS sequences to date, two cases were technically suboptimal and are excluded from this report. The suboptimal cases and the single-voxel cases led to development of the highly selective saturation bands needed to decrease artifacts from the heart/chest wall and in large breasts, and also led to further improvements in the multi-voxel sequences. Furthermore, use of the initial single-voxel cases validated use of the contrast-enhanced breast MR images to use as a guide for correlating the location of the tumor within the breast to the tumors biopsied under direct ultrasound visualization for fine-needle sampling. Lastly, use of the single-voxel and multi-voxel sequences in the same patient helped to develop and confirm presence of choline in the same location within the breast (Appendix 1).

Using correction factors obtained from the pre-scan transmitter gain and by optimally combining data from each of the coils, we were able to obtain quantitative measurements of choline levels within the breast lesions during the MRI/MRS scan. Subsequent to each MRI/MRS study, all lesions underwent tissue aspiration from suspicious breast masss

using a 21 gauge needle (4 needle passes in 7 patients, 6 needle passes in 2 operated breast specimens). Direct ultrasound guidance was used to direct the needle to obtain cell samples in 7 patients immediately after the scan. In 2 patients, aspirates were taken directly from breast tissue in the operating room. In these 2 cases the needle was directed into the mass for cell removal by physician palpation immediately upon tissue removal during surgery. Three patients had previously undergone both chemotherapy and radiation therapy and had masses suspicious for breast cancer recurrence and 6 patients had neither chemotherapy nor radiation therapy.

To date, 5 patients have had biopsy proof and 4 are awaiting surgery. In the 5 patients with biopsy proof, the *in vivo* MRS studies showed elevated choline peaks in 2 pre-treatment patients with breast cancer, no choline peak in two patients with benign disease (1 biopsy scar post-chemotherapy/radiation, 1 fibroadenoma) and an elevated choline peak in 1 post-treatment breast cancer patient who had a breast cancer recurrence after undergoing chemotherapy and radiation therapy. The *in vitro* MRS studies of the FNA breast cell samples showed elevated choline peaks in the 2 untreated breast cancers, and no choline in the breast scar, the post-treatment breast cancer recurrence or the fibroadenoma. Elevated *in vitro* MRS choline peaks correlated well with elevated choline peaks seen on the *in vivo* MRS data on the 2 untreated breast cancers. Absence of an elevated choline peak in both the *in vitro* and *in vivo* MRS scans also correlated with the breast scar and the fibroadenoma. However, we expected elevated choline peaks in the recurrent cancer in the post-treatment cancer patient but only detected an elevated choline peak elevation on the *in vivo* MRS study; the *in vitro* MRS scan unexpectedly showed no choline peak in the cell samples. We speculate that absence of the *in vitro* MRS choline peak might be due to fine-needle aspiration sampling error during the breast needle biopsy. Non-viable regions of breast cancer after chemotherapy and radiation therapy have a similar appearance to living breast cancers on breast ultrasound. We had used breast ultrasound to guide the biopsy needle into the tumor to obtain material for *in vitro* MRS analysis. Thus, absence of *in vitro* MRS choline peaks in the breast cell samples in our post-treatment patient might be due to sampling of a necrotic part of the tumor.

KEY RESEARCH ACCOMPLISHMENTS

1) Development and optimization of high resolution *in vitro* MRS to analyze human cell samples for choline.
2) Development and optimization of the *in vivo* multi-voxel MRS pulse sequences to detect choline by redesigning RF pulses for improved lipid and water suppression using dual BASING techniques.
3) Addition of highly selected saturation bands used to suppress tissue giving rise to artifacts (chest wall/ heart, large breasts near the RF coil)
4) Validation of use of MRI images to correlate location of tumors for *in vivo* multi-voxel MRS and subsequent fine-needle biopsies Development of personnel infrastructure and protocols to enable recruitment of normal volunteers and cancer patients for MRI/MRS scans and fine-needle biopsies.

5) Testing and further development of optimized *in vivo* multi-voxel MRS pulse sequences and high resolution *in vitro* MRS of human breast cells obtained by fine-needle aspiration on patients.

REPORTABLE OUTCOMES

Abstracts:

Spielman D, Hunjan S, Ikeda DM, Adalsteinsson E, Sawyer-Glover AM. Proton spectroscopic imaging of breast cancer. Submitted to International Society for Magnetic Resonance in Medicine 9th Scientific Meeting, Glasgow, Scotland, April 21-27, 2001

Hunjan S, Spielman D, Ikeda DM, Adalsteinsson E, Sawyer-Glover AM. Comparison of in vivo and in vitro MRS of breast cancer and breast disease. Submitted to International Society for Magnetic Resonance in Medicine 9th Scientific Meeting, Glasgow, Scotland, April 21-27, 2001

Journal Articles:

Star-Lack JM, Adalsteinsson E, Gold GE, Ikeda DM, Spielman DM. Motion correction and lipid suppression for 1H magnetic resonance spectroscopy. Magn Reson Med, 2000. 43 (3): p. 325-330.

Degrees Supported by this Award:

Postdoctoral Research Affiliate: Sandeep Hunjan, Ph.D.

CONCLUSIONS:

The overall objective of this project is to use MRS in the diagnosis and therapy planning of breast tumors detected by contrast-enhanced breast MRI by providing spectroscopic information of key metabolites found in breast lesions. Technical developments to date have significantly contributed towards the goal of making MR spectroscopic imaging a clinically useful procedure, particularly for untreated breast cancers. Specific applications of this work include imaging of choline for the differentiation of breast cancer from benign breast masses, and radiation necrosis from breast conserving therapy versus tumor recurrence.

Plans: During the next year, we will continue the development of volumetric spectroscopic imaging techniques with emphasis on acquiring short-echo-time *in vivo* proton spectra in order significantly increase the signal to noise ratio for observable metabolites. We plan to decrease the voxel size and obtain more acquisitions for improved tumor metabolite signals, particularly in treated breast cancers. Additional investigations will focus on performing more *in vivo* and in vitro spectroscopy of breast carcinomas and benign tumors, and focus on increasing the yield of breast cell samples from post-treatment cancer patients.

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Appendix 1

Images from Magnetic Resonance Imaging studies of a woman with a large untreated breast cancer, and corresponding in vivo and in vitro spectroscopy data showing choline peaks expected in breast cancer, and not present in normal breast tissue.

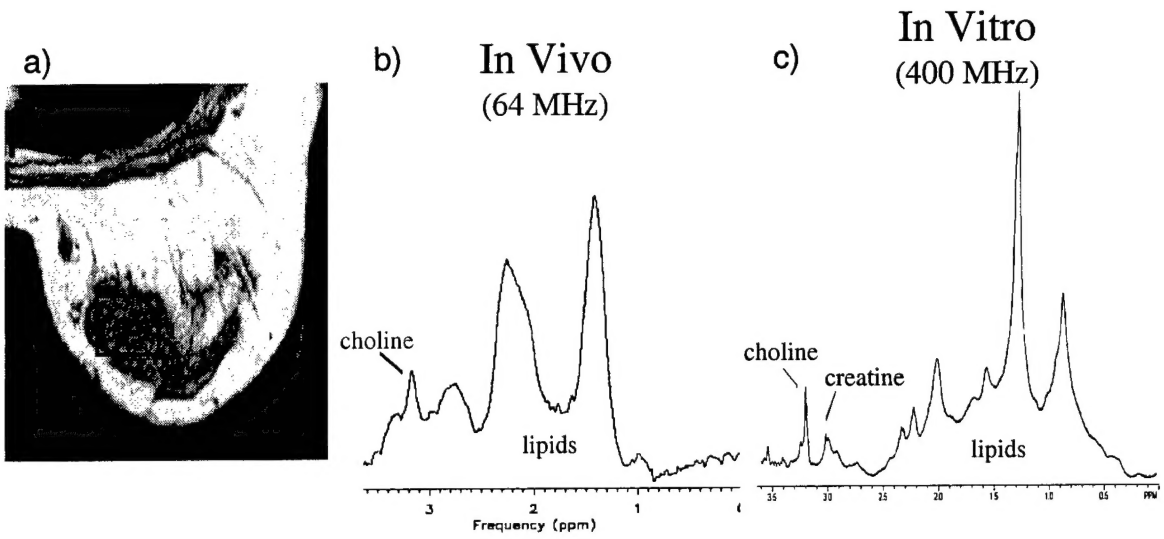

Figure 1. Single voxel MRS of breast cancer of a woman with a large invasive ductal cancer. (a) T1-weighted image showing location of 3x3x3 cm^3 spectroscopic voxel. (b) spectrum corresponding to voxel shown in (a) collected with the following parameters: PRESS localization, TR/TE = 2000/144 ms, 64 averages, water and lipid suppression via BASING technique. (c) 400 MHz in vitro spectrum from fine needle aspirate of the lesion shown in (a) (TR=4s, 1024 FIDs). Note, no lipid suppression is used in (c) in contrast to the in vivo data (b) in which saturation pulses partially suppress the 1.3 and 0.9 ppm lipid peaks. Choline (3.2 ppm) is visible in both in vivo and in vitro spectra.

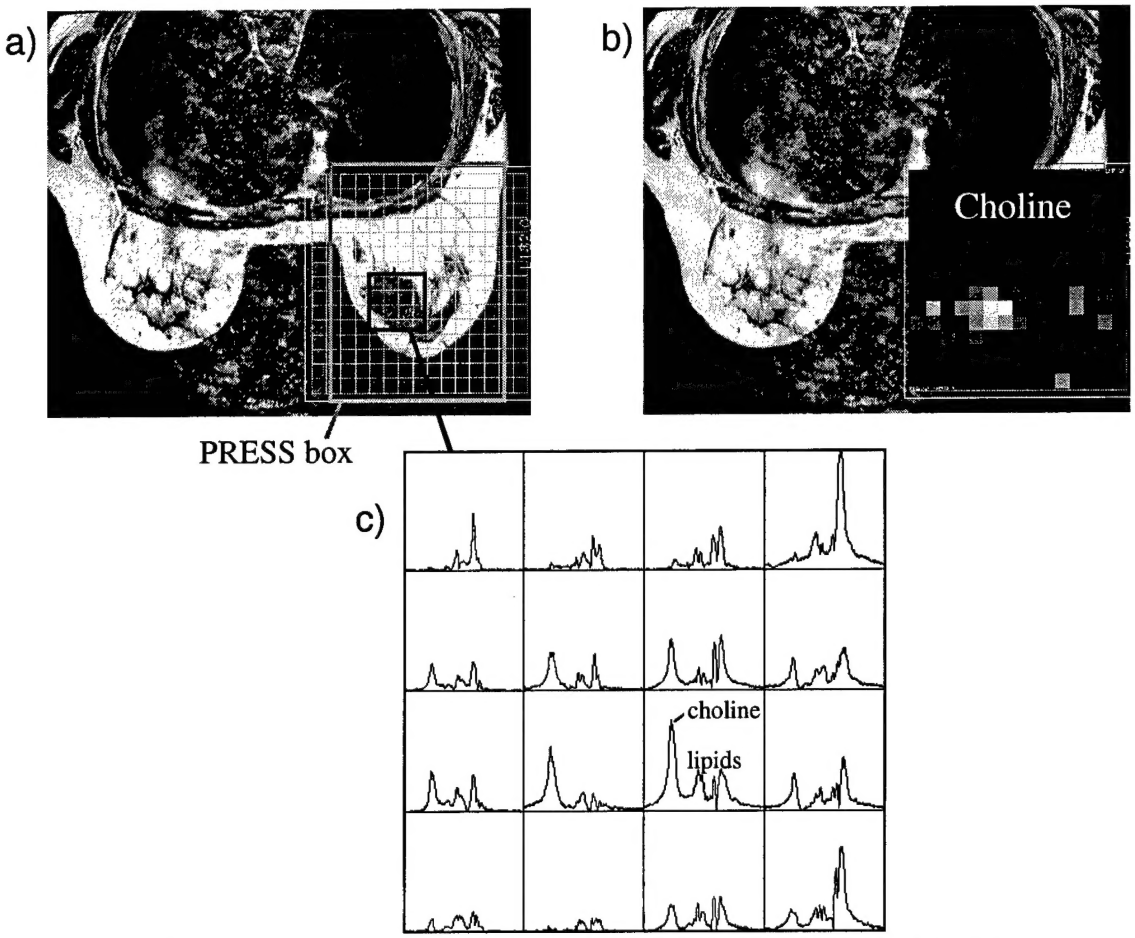

Figure 2. ^{1}H spectroscopic imaging of breast cancer. (a) T1-weighted imaging showing PRESS box and corresponding 16x16 array of 1cc voxels. (b) metabolic map of the choline peak overlayed on the T1-weighted image. (c) spectra from subset of voxels highlighted in (a). High choline is visible within the lesion.